(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 July 2004 (15.07.2004)

PCT

(10) International Publication Number
WO 2004/058976 A3

(51) International Patent Classification[7]: C12N 15/52, C07D 313/00, C12P 17/08, C12R 1/465

(21) International Application Number: PCT/GB2003/005704

(22) International Filing Date: 24 December 2003 (24.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0230217.2 27 December 2002 (27.12.2002) GB

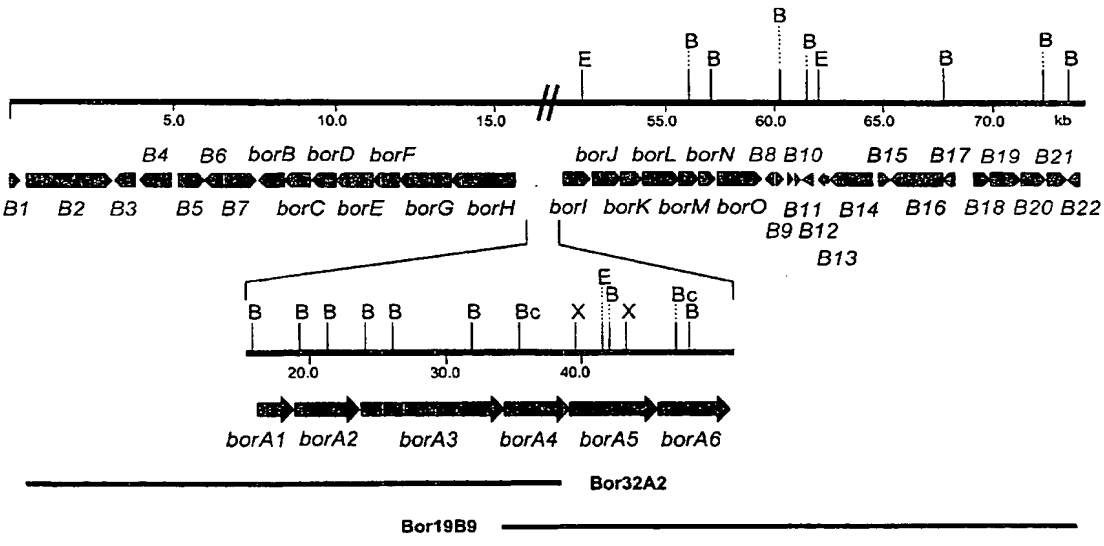
(71) Applicants *(for all designated States except US)*: **BIOTICA TECHNOLOGY LIMITED** [GB/GB]; Chesterford Research Park, Little Chesterford, Nr Saffron Walden, Exxex CB10 1XL (GB). **THE UNIVERSITY OF OVIEDO** [ES/ES]; San Francisco 3, E-33003 Oviedo (ES).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SALAS, Jose, A.** [ES/ES]; The University of Oviedo, Departamento de Biologia Funcionale, Instituto Universitario de Oncologia del, Principado de Asturia (I.U.O.P.A), Universidad de Oviedo, E-33006 Oviedo (ES). **MENDEZ, Carmen** [ES/ES]; The University of Oviedo, Departamento de Biologia Funcionale, Instituto Universitario de Oncologia del, Principado de Asturia (I.U.O.P.A), Universidad de Oviedo, E-33006 Oviedo (ES). **OLANO, Carlos** [ES/ES]; University of Oviedo, Departamento de Biologia Funcionale, Instituto Universitario de Oncologia del, Principado de Asturia (I.U.O.P.A), Universidad de Oviedo, E-33006 Oviedo (ES). **SANCHEZ, Cesar** [ES/ES]; The University of Oviedo, Departamento de Biologia Funcionale, Instituto Universitario de Oncologia del, Principado de Asturia (I.U.O.P.A), Universidad de Oviedo, E-33006 Oviedo (ES). **BRANA, Alfredo, F.** [ES/ES]; The University of Oviedo, Departamento de Biologia Funcionale, Instituto Universitario de Oncologia del, Principado de Asturia (I.U.O.P.A), Universidad de Oviedo, E-33006 Oviedo (ES). **WILKINSON, Barrie** [GB/GB]; Biotica Technology Limited, Chesterford Research Park, Little Chesterford, Nr Saffron Walden, Essex CB10 1XL (GB). **MARTIN, Christine, J.** [GB/GB]; Biotica Technology Limited, Chesterford Research Park, Little Chesterford, Nr Saffron Walden, Essex CB10 1XL (GB). **MOSS, Steven** [GB/GB]; Biotica Technology Limited, Chesterford Research Park, Little Chesterford, Nr Saffron Walden, Essex CB10 1XL (GB). **LEADLAY, Peter, F.** [GB/GB]; Biotica Technology Limited, Chesterford Research Park, Little Chesterford, Nr Saffron Walden, Essex CB10 1XL (GB). **OLIYNYK, Marko** [UA/GB]; Department of Biochemistry, University of Cambridge,

[Continued on next page]

(54) Title: BORRELIDIN-PRODUCING POLYKETIDE SYNTHASE AND ITS USE



(57) Abstract: The present invention relates to the biosynthesis of polyketides and derives from the cloning of nucleic acids encoding a polyketide synthase and other associated proteins involved in the synthesis of the polyketide borrelidin. Materials and methods including enzyme systems, nucleic acids, vectors and cells are provided for the preparation of polyketides including borrelidin and analogues and derivatives thereof. Novel polyketide molecules are also provided.

WO 2004/058976 A3

80 Tennis Court Road, Cambridge, Cambridgeshire CB2 1GA (GB).

(74) **Agents: FORREST, Graham, R.** et al.; Mewburn Ellis, York House, 23 Kingsway, London, Greater London WC2B 6HP (GB).

(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *with international search report*

(88) Date of publication of the international search report:
17 February 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/GB 03/05704

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/52 C07D313/00 C12P17/08 C12R1/465

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07D C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BENTLEY S D ET AL: "Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2)" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 417, no. 6885, 2002, pages 141-147, XP002233530 ISSN: 0028-0836 Sequences Tr-EMBL accession numbers SC0939106, Q9RCV7, Q9RJG7, Q9RJG8, Q9RJG9, Q9RJG5, Q9RJG6 & Q9ZBH2 ----- -/--	1,2,18, 52-56

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2004	22.10.04

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Page, M

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Ir tional Application No
PCT/GB 03/05704

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BANTLEON R ET AL: "CHLOROPEROXIDASE FROM STREPTOMYCES LIVIDANS: ISOLATION AND CHARACTERIZATION OF THE ENZYME AND THE CORRESPONDING GENE" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 176, no. 8, 1 April 1994 (1994-04-01), pages 2339-2347, XP000560163 ISSN: 0021-9193 Sequence EMBL accession number SLPOL	1,2
A	DE 36 07 287 A (BAYER AG) 7 January 1988 (1988-01-07) page 3, line 43 - line 45	1-42, 52-57
A	REEVES CHRISTOPHER D ET AL: "Alteration of the substrate specificity of a modular polyketide synthase acyltransferase domain through site-specific mutations" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 40, no. 51, 25 December 2001 (2001-12-25), pages 15464-15470, XP002194845 ISSN: 0006-2960 the whole document	19-39
A	WO 01/68867 A (LEADLAY PETER FRANCIS ;OLIYNYK MARKO (GB); STAUNTON JAMES (GB); BI) 20 September 2001 (2001-09-20) cited in the application page 1, line 25 - page 12, line 12; figures 3,4	1-42, 52-57
A	SWAN D G ET AL: "Characterisation of a Streptomyces antibioticus gene encoding a type I polyketide synthase which has an unusual coding sequence" MOLECULAR AND GENERAL GENETICS, SPRINGER VERLAG, BERLIN, DE, vol. 242, no. 3, 1994, pages 358-362, XP002087278 ISSN: 0026-8925 cited in the application the whole document	

INTERNATIONAL SEARCH REPORT

Iernational application No.
PCT/GB 03/05704

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 2 ,17, 18, 42, 52-57 (partially) and 3-16, 19-41 (completely)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 17, 18, 42, 52-57 (partially) and 3-16, 19-41 (completely)

Invention 1: Borrelidin polyketide synthase enzyme according to SEQ ID NOs. 2-7, polynucleotide encoding the same, method of using the same to modify a further polyketide synthase or modifying the same using a further polyketide synthase, method of increasing the capacity of a host cell to produce borrelidin by upregulating the expression of the same, vectors and host cells.

---

2. claims: 1, 2, 17, 18, 42-57 (partially)

Invention 2: Enzyme involved in the biosynthesis of borrelidin according to SEQ ID NOs. 8-21, polynucleotide encoding the same, method of using the same to modify a further polyketide synthase or modifying the same using a further polyketide synthase, method of increasing the capacity of a host cell to produce borrelidin by upregulating the expression of the same, vectors and host cells.

---

3. claims: 1, 2, 17, 18, 42-57 (partially)

Inventions 3-25: Enzyme involved in the biosynthesis of borrelidin according to SEQ ID NOs. 22-43 and 113 respectively, polynucleotide encoding the same, method of using the same to modify a further polyketide synthase or modifying the same using a further polyketide synthase, method of increasing the capacity of a host cell to produce borrelidin by upregulating the expression of the same, vectors and host cells.

---

4. claims: 58-73

Inventions 26-31: Borrelidin-like molecule according to claim 58 or 59 substitured at at least position R1-R13 respectively.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/GB 03/05704

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3607287 A	07-01-1988	DE 3607287 A1	07-01-1988
WO 0168867 A	20-09-2001	AU 4940800 A	24-09-2001
		EP 1183369 A1	06-03-2002
		WO 0168867 A1	20-09-2001